VERIFICATION OF A TRANSLATION

I, the below named translator, hereby declare that:

My name and post office address are as stated below:

That I am knowledgeable in the English language and in the language in which the below identified French Application was filed, and that I believe the English translation of the French Application N° 9507007 filed on 13/06/95 in the name of Institut Pasteur

is a true and complete translation of the above identified French Application as filed.

Date: ....28/04/2000....

Full name of the translator — Véronique MARCADÉ

Signature of the translator

Post Office Address — 3, rue Chauveau-Lagarde
F-75008 Paris
FRANCE

POLYPEPTIDE MOLECULES OF THE PRE-ERYTHROCYTIC STAGE OF MALARIA

The parasites responsible for malaria in man display different morphologies in the human host and express different antigens depending on their location in the body. The morphological and antigenic differences of these parasites during their life cycles in man enable different stages of development in the liver and in the blood to be defined: the sporozoite, the infectious form injected by the vector mosquito, transforms rapidly into a schizont in the host's hepatocytes and thereafter infects the erythrocytes. The intrahepatic localization of P.falciparum manifests itself in the expression of a group of antigens specific to this stage of development and which are highly immunogenic under the natural conditions of exposure to the disease. This clinically silent phase is at present the only one against which a very strong, sterilizing immunity can be induced experimentally in man, by injecting irradiated sporozoites capable of entering the hepatocyte and of developing therein but without being able to lead on to the blood stage of the disease. Accordingly, the inventors have concentrated the bulk of their efforts on these two pre-erythrocytic stages. However, these stages are also the most intricate ones to study, and hence the least understood, since it is difficult or even impossible to obtain biological material, the only in vitro study

model affords a very low yield and the best animal model remains the chimpanzee, the use of which is limited and expensive.

In order to gain access to the antigens of the pre-erythrocytic stages, the inventors used sera of individuals who had resided for 25 years in a region where the disease is endemic but who were on permanent prophylaxis with chloroquine. These individuals were regularly subjected to infected mosquito bites but did not develop any complete blood infection. Their serum hence contained antibodies directed essentially against the pre-erythrocytic stages, which was verified by immunofluorescence (IF) and western blotting on the 3 stages of the parasite.

The use of these sera for screening a library of genomic DNA of the parasitic clone of P.falciparum, the library being constructed in expression vectors in a phage lambda gt11 (V. Rosario, Science 212, 1981, pp. 1037-1038; and Thaithong et al., Transactions of Royal Society of Tropical Medicine and Hygiene, 1984, 78:242-245), led to the demonstration of polypeptides of the pre-erythrocytic stage, in particular the SALSA (sporozoite liver stage antigen) polypeptides described in EP A-0,407,230 and LSA-1 (liver stage antigen) described in WO 92/13884. The present invention relates to new polypeptide molecules specific to the pre-erythrocytic stage, and to their use as active principle of antimalarial vaccine or in methods of diagnosis of the disease.

The invention is the outcome of the demonstration by the inventors of the special properties of a particular antigen referred to LSA-3 and of its fragments, which are seen to be candidates with a strong potential for producing an antimalarial vaccine, for the following reasons:

a) when a fraction of LSA-3 was used in combination with another antigen of the same stage of development of the parasite, such as LSA-1, to immunize

chimpanzees, the animal responding to both molecules or only to LSA-3 displays the feature of not having parasites in the blood, of having a substantial decrease of the parasites in the liver and of manifesting a substantial recruitment of mononuclear cells indicating a response in terms of cellular immunity;

b) in regions where the disease is endemic, a very clear correlation is observed between the protection of individuals against natural infection by sporozoites and their responses in terms of antibodies against LSA-3;

c) in eight human volunteers immunized by injection of irradiated sporozoites, antibodies directed against LSA-3 are found in each of the four individuals resisting sporozoite infection and in none of the other four volunteers who developed a blood infection;

d) antibodies obtained against the peptide DG729 in WO 92/13884, already described, give a cross-reaction with the sporozoite and liver stages of the murine parasite P.yoelii, which permits a significant exploitation of the mouse model. In vitro, the human antibodies immunopurified on DG729 are capable, even at very low concentrations, of blocking the entry of P.yoelii sporozoites into mouse hepatocytes. In vivo, mice immunized with DG729 are fully or partially protected against infection by P.yoelii sporozoites;

e) lastly, some epitopes, in particular in the non-repeat portions of the molecule, stimulate the secretion of interferon-γ by monocytes, this mediator enabling the intrahepatic development of the parasite to be inhibited (S. Mellouk et al., The Jour. Of Immun. 139, 4192-4195, 1987);

All these properties, some of which will be demonstrated in detail in the experiments described later, show that the LSA-3 antigen displays both good antigenicity and good immunogenicity.

The inventors were able to confirm and define the specificity of the stages of expression of the

molecule; in the sporozoites, this expression was confirmed by the surface immunofluorescence of several strains and isolates. In Western Blot analysis, the LSA-3 molecule appears as a protein of molecular weight 200,000 daltons. While the messenger RNAs of sporozoites could not be obtained in sufficient amounts for a northern blot analysis, reverse PCR experiments confirmed the expression of LSA-3 at this stage. In infected hepatocytes, LSA-3 is observed in the parasitophorous vacuole of the parasite by immunofluorescence using antibodies against the repeat and non-repeat regions of the protein, as well as by electron microscopy.

A fragment of LSA-3 designated 729S, as well as three peptides designated NRI and NRII included in the non-repeat portion and 729R included in the repeat portion, have been described in Application WO 92/13884. Nevertheless, this document does not mention the special properties mentioned above, or other fragments of LSA-3 which could be either longer or shorter, included or combined with these fragments, which might display especially advantageous properties for use in vaccines.

The subject of the invention is polypeptide molecules containing at least ten consecutive amino acids of the amino acid sequence shown in Figure 2 and designated SEQ ID No. 2, and representing LSA-3, the following polypeptides being excluded:

- RDELFNELLNSVDVNGEVKENILEESQVNDDIFNSLVKSVQQEQQHNVEE
- VEESVEENDEESVEENVEENVENNDDGSVASSVEESIASSVDESIDSSIE-ENVAPTVEEIVAPTVEEIVAPSVVEKCAPSVEESVAPSVEESVAEMLKER (729S)
- RDELFNELLNSVDVNGEVKENILEESQVNDDIFNSLVKSVQQEQQHN
- DELFNELLNSVDVNGEVKENILEESQ, (NRI)
- LEESQVNDDIFSNSLVKSVQQEQQHNV, (NRII)
- VESVAPSVEESVAPSVEESVAENVEESV. (729RE)

Other molecules according to the invention contain at least 20 consecutive amino acids or at least 50.

This set of polypeptides and the LSA-3 molecule are, throughout hereinafter, "polypeptides of the invention".

The experimental results and the comparisons of sequences between different P.falciparum isolates indicate the existence of at least 70% homology between equivalent antigens of the liver stage of the parasite. Thus any peptide molecule displaying at least 70% homology with any one of the molecules defined above forms part of the invention, as do those displaying at least 70% homology with the following sequence:

Leu Leu Ser Asn Ile Glu Glu Pro Lys Glu Asn Ile Ile Asp Asn Leu Leu Asn Asn Ile (CT1)

lying between amino acids 140 and 159 of K1 or 23 and 42 of T9/96.

Likewise forming part of the invention are the polypeptide molecules displaying at least 70% homology with the sequence depicted in Figure 3, which depicts a portion of LSA-3 in T9/96: the DNA of this P.falciparum isolate was digested with restriction enzymes, then cloned into lambda gt11 and thus enabled the gene library of this isolate, already described above, to be constituted.

Conjugates consisting of a polypeptide originating from LSA-3 linked covalently via a lysine bridge to saturated or unsaturated lipid residues also form part of the invention, more especially when the lipid residue is a palmitoyl or a palmityl or an oleyl. C_{16} or C_{18} residues were thus coupled via a lysine bridge to the peptides NRI, NRII, 729RE and CT1 already depicted above. The method of synthesis used for these conjugates is described in Bourgault, Journal of Immunology, 149, 3416 (1992) and Rouaix, Vaccine, 12, 1209 (1994).

The invention also covers immunogenic compositions containing at least one polypeptide molecule or one conjugate described above, as well as the vaccines containing these immunogenic compositions. Other immunogenic epitopes, in particular LSA-1, SALSA, STARP, have already been described in EP A-0407230 and in WO 92/13884. The vaccine compositions according to the invention can advantageously contain a mixture of immunogenic peptides originating from LSA-3 and of the peptides or antigens originating from LSA-1, SALSA or STARP; a more especially advantageous mixture could be the one consisting, on the one hand of NRI, NRII or whole LSA-3, these being coupled or otherwise to a lipid residue, and on the other hand the peptides SALSA-1, SALSA-2 or the SALSA antigen coupled or otherwise to a lipid residue.

All polypeptide molecules corresponding to the above definition and displaying at least 70% homology with the polypeptides LSA-3, CT1, NRI, NRII or 729RE may be combined in homologous or heterologous fashion with other peptide sequences or sequences originating from another antigen of the different stages of P.falciparum.

The invention also covers the polyclonal or monoclonal antibodies which specifically recognize the polypeptide molecules of the invention.

These molecules of the invention may be used for carrying out diagnostic methods and producing kits enabling the existence of P.falciparum infection to be detected; this method can be either an assay of circulating specific antibodies, by carrying out standard serological methods by bringing one of the above antigens into contact with a biological fluid of the individual in question, or methods of assay of antigens using polyclonal or monoclonal antibodies obtained by standard methods for obtaining such antibodies with the corresponding antigens. In the diagnostic outfits or kits of the invention, the

reagents enabling the antigen/antibody complexes produced to be detected, which can also carry a label or be capable of being recognized in their turn by a labelled reagent, are present. Depending on whether it is desired to carry out an antigen test or a serological test, the kit comprises either the antibodies or the antigens of the invention.

The invention also covers all the nucleotide sequences coding for a polypeptide of the invention, as well as any recombinant nucleic acid containing at least one nucleotide sequence of the invention, inserted into a nucleic acid which is heterologous with respect to the said nucleotide sequence.

The nucleic acid sequences coding for LSA-3 or its immunogenic fragments and corresponding to one of the following definitions form part of the invention:

(a) the linked succession of nucleotides as depicted in SEQ ID No. 1 of Figure 1, or

(b) the linked succession of nucleotides depicted in SEQ ID No. 2 of Figure 2,

(c) a linked succession displaying at least 70% homology with that of Figure 1 or of Figure 2, or

(d) a linked succession of nucleotides which are complementary to those presented in (a), (b) or (c).

The expression "coding for LSA-3" is understood to refer both to the gene depicted in SEQ ID No. 1 of Figure 1 and the cDNA depicted in SEQ ID No. 2 of Figure 2.

The invention relates more especially to a recombinant nucleic acid in which the nucleotide sequence of the invention is preceded by a promoter (in particular an inducible promoter), under the control of which the transcription of the said sequence is capable of being performed, and, where appropriate, followed by a sequence coding for transcription termination signals.

The invention also covers the coding sequence originating from the clone T9/96 depicted in Figure 3 by SEQ ID No. 3.

In this sequence, the fragment CT1 lies between nucleotides 67 and 126, the fragment 679 begins at nucleotide 206 and the fragment 729RE lies between nucleotides 547 and 630.

Lastly, the invention covers any recombinant vector used especially for the cloning of a nucleotide sequence of the invention, and/or for the expression of the polypeptide encoded by this sequence, and characterized in that it contains a recombinant nucleic acid as defined above in one of its sites which is not essential for its replication.

As an example of an abovementioned vector, plasmids, cosmids, phages or viruses may be mentioned.

As such, the invention relates more especially to the plasmid pK 1.2. deposited at the CNCM under the No. I-1573.

The subject of the invention is also a method for preparing a polypeptide of the invention, by transformation of a cell host using a recombinant vector of the abovementioned type, followed by the culturing of the cell host thus transformed and the recovery of the polypeptide in the culture medium.

Thus, the invention relates to any cell host transformed by a recombinant vector as defined above, and comprising the regulatory elements permitting the expression of the nucleotide sequence coding for a polypeptide according to the invention.

The invention likewise covers DNA (or RNA) primers which can be used in the context of the synthesis of nucleotide and/or polypeptide sequences of the invention, by the PCR (polymerase chain reaction) technique or any other method known at the present time for amplifying nucleic acids, such as LCR, CPR, ERA, SPA, NASBA, and the like.

The invention relates to any DNA or RNA primer, characterized in that it consists of approximately 10 to 25 nucleotides which are identical or complementary to the first 10 to 25 nucleotides of the nucleotide sequence coding for a peptide sequence according to the invention, or identical to the last 10 to 25 nucleotides of the said sequence.

Thus, the present invention also covers a method for preparing a polypeptide of the invention comprising the following steps:

- where appropriate, the prior amplification by standard techniques of the amount of nucleotide sequences coding for the said polypeptide using two suitably chosen DNA primers,
- the culturing, in a suitable culture medium, of a cell host previously transformed by a vector containing a nucleic acid according to the invention comprising the nucleotide sequence coding for the said polypeptide, and
- the recovery from the abovementioned culture medium of the polypeptide produced by the said transformed cell host.

By way of example of DNA or RNA primers according to the invention, the following pairs of sequences may be mentioned:

S1: GTGATGAACTTTTTAATGAATTATTAAA (SEQ ID No. 4)

S2: TGTTGTTCTTGTTGAACACTTTTTACTAA (SEQ ID No. 5)

whose respective positions on the LSA-3/K1 gene depicted in Figure 1 are from 695 to 722 and from 829 to 799 (reading in the reverse direction), or the pair:

6.1: GGTATCGAAACTGAGGAAATAAAGG (SEQ ID No. 6)

6.2: CATAGCAGGAACATCAACATCCAC (SEQ ID No. 7)

whose respective positions are 2668 to 2692 for 6.1 and 3456 to 3433 for 6.2 (reading in the reverse direction).

The peptides of the invention may also be prepared by the standard techniques of peptide synthesis. This synthesis may be carried out in

homogeneous solution or in the solid phase. For example, use may be made of the technique of synthesis in homogeneous solution described by Houben-Weyl in the work entitled "Methoden der Organischen Chemie" (Methods in Organic Chemistry) edited by E. Wunsch, vol. 15-I and II. Thieme, Stuttgart 1974, or that described by R.D. Merrifield in the paper entitled "Solid phase peptide synthesis" (J. Am. Chem. Soc., 45, 2149-2154).

The invention also covers the water-soluble oligomers of the abovementioned monomeric peptides.

Oligomerization can cause an enhancement of the immunogenicity of the monomeric peptides according to the invention. While such numerical information cannot be regarded as limiting, it may nevertheless be mentioned that these oligomers can, for example, contain from 2 to 10 monomer units.

To carry out the oligomerization, use may be made of any polymerization technique commonly used in the peptide field, this polymerization being conducted until an oligomer or polymer containing the requisite number of monomer motifs for acquiring the desired immunogenicity is obtained.

One method of oligomerization or polymerization of the monomer consists in reacting the latter with a crosslinking agent such as glutaraldehyde.

Use may also be made of other oligomerization or coupling methods, for example the one employing successive couplings of monomer units via their carboxy- and amino-terminal functions in the presence of homo- or heterobifunctional coupling agents.

The invention also relates to the conjugates obtained by covalent coupling of the peptides according to the invention (or of the abovementioned oligomers) to physiologically acceptable and non-toxic (natural or synthetic) carrier molecules that enable, in particular, the immunogenicity to be increased, via complementary reactive groups carried, respectively, by

the carrier molecule and the peptide. By way of example of macromolecular carrier molecules or supports which participate in the constitution of the conjugates according to the invention, there may be mentioned natural proteins such as tetanus toxoid, ovalbumin, serum albumins, haemocyanins, tuberculin PPD (PPD: purified protein derivative), and the like.

By way of synthetic macromolecular supports, there may be mentioned, for example, polylysines or poly(DL-alanine)-poly(L-lysine)s.

By way of hydrocarbon or lipid supports, there may be mentioned saturated or unsaturated fatty acids, and preferably C_{16} or C_{18} acids of the oleic or palmitoleic type.

To synthesize the conjugates according to the invention, use may be made of methods which are known per se, such as the one described by Frantz and Robertson in Infect. and Immunity, 33, 193-198 (1981), or the one described in Applied and Environmental Microbiology (October 1981), vol. 42, No. 4, 611-614 by P.E. Kauffman, using the peptide and the appropriate carrier molecule.

The nucleic acids of the invention may be prepared either by a chemical method or by other methods.

A suitable method of preparing the nucleic acids of the invention containing not more than 200 nucleotides (or 200 bp in the case of double-stranded nucleic acids) comprises the following steps:

- DNA synthesis using the automated β-cyanoethyl-phosphoramidite method described in Bioorganic Chemistry 4; 274-325 (1986),
- cloning of the nucleic acids thereby obtained into a suitable vector and recovery of the nucleic acid by hybridization with a suitable probe.

A chemical method of preparation of nucleic acids of length greater than 200 nucleotides has already been described in WO 92/13884.

The invention also relates to diagnostic kits which contain one or more amplification primers specific for the LSA-3 gene and which enable the presence of the gene or of the mRNA to be detected in an individual likely to be infected by P.falciparum.

The invention also covers pharmaceutical or vaccine compositions in which at least one of the products according to the invention is present in combination with solid or liquid, pharmaceutically acceptable excipients suitable for the construction of oral, ocular or nasal dosage forms, or excipients suitable for the construction of dosage forms for rectal administration, or alternatively with gelatinous excipients for vaginal administration. It also relates to isotonic liquid compositions containing at least one of the conjugates according to the invention, suitable for administration to the mucosae, in particular the ocular or nasal or pulmonary mucosae.

Advantageously, the vaccine compositions according to the invention contain, in addition, a vehicle such as polyvinylpyrrolidone which facilitates the administration of the vaccine. In place of polyvinylpyrrolidone, it is possible to use any other type of adjuvant, in the traditional sense which was formerly given to this expression, that is to say a substance which enables a medicinal product to be absorbed more readily or which facilitates its action in the body. By way of examples of other adjuvants of this latter type, there may also be mentioned carboxymethylcellulose, aluminium hydroxides and phosphates, saponin or all other adjuvants of this type which are well known to a person skilled in the art. Lastly, they contain, if necessary, an immunological adjuvant, in particular of the muramyl peptide type.

The invention also relates to pharmaceutical compositions containing as active substance at least one of the polyclonal or monoclonal antibodies defined

above, in combination with a pharmaceutically acceptable vehicle.

Additional features of the invention will also become apparent in the examples illustrated with the figures which follow, and show the special features of the molecules of the invention relative to other antigens of the pre-erythrocytic stage of the parasite.

Figure 1 depicts the genomic DNA sequence ID No. 1 of 6152 base pairs of the LSA-3 gene; it originates from the clone K1.2, which itself originates from a Thai isolate.

Figure 2 depicts the cDNA sequence ID No. 2 and the polypeptide sequence of the LSA-3 antigen. The DNA sequence represents 5361 base pairs.

Figure 3 depicts the sequence ID No. 3 of the portion sequenced in the parasite clone T9/96 (1890 base pairs), the upper line being the nucleotide sequence and the lower line the peptide sequence. In this clone, the CT1 sequence lies between nucleotides 67 and 126, the actual fragment DG679 beginning at nucleotide 207. The fragment 729RE lies between nucleotides 547 and 629.

Figure 4a depicts diagrammatically the relative positions of the repeat and non-repeat sequences, the introns and the exons in strains K1 and T9/96, the clones 679 and 729 originating from the latter.

Figure 4b depicts the HCP (hydrophobic cluster plot) of the peptide sequence of the clone DG729.

Figure 5 depicts the amounts of immunoglobulins produced in the serum of chimpanzee Nuria before and after immunization with different LSA-3 peptides.

Figure 6 shows the specific antibody titre of different species of mice immunized either with a peptide or with a corresponding lipopeptide.

Figure 7 shows the inhibition of the sporozoite invasion of liver cells by hyperimmune sera obtained after immunization with different peptides and immunopurified against whole LSA-3.

Figure 8 depicts the comparison of an antigen originating from LSA-3 with two other antigens with respect to type T immunity.

Figure 9 depicts the induction of interferon-γ in the chimpanzees Gerda and Dirk with the peptides originating from the LSA-3 molecule.

Example 1: Cloning and sequencing of the LSA-3 gene

1) Sequencing

Initial screening of the gene library originating from the parasite clone T9/96 with the serum of a missionary treated continuously by prophylaxis enabled us to isolate 120 clones corresponding to molecules expressed at the sporozoite and/or liver stage of the P.falciparum cycle. The clone 729S was used as probe to screen a genomic library of the Thai strain K1 already mentioned above, which contains large EcoR I fragments cloned into phage lambda gt10. A 6.85-kilobase insert containing the whole gene was purified from this gene library and recloned into a pUC18 plasmid for sequencing and characterization. In P.falciparum, the genome of which is very rich in bases A:T(80%), this approach is often rendered difficult by the rarity of restriction sites which can be used, and by the instability or even the impossibility of cloning certain fragments when they are inserted into plasmid vectors.

The structure of the gene is depicted in Figure 4 and displays the following features:

a) a mini-exon 1 coding at its 3' end for a hydrophobic signal peptide;

b) a short intron (168 base pairs) included between consensus splicing donor and acceptor sites;

c) a second exon of five kilobases which codes for an organized region of 1.8 kilobases, and composed of an arrangement of 7 blocks of 4 amino acids and a 3' hydrophobic region which might correspond to an

anchorage of the glycosylphosphatidylinositol (GPI) type.

A detailed investigation of the polymorphism of LSA-3 was carried out by sequencing the clone 679, which contains the bulk of the repeat sequences of the LSA-3 gene and a 1-kilobase portion of the 3' non-repeat fraction, the sequence of this fragment being depicted in Figure 3 between nucleotides 207 and 1890.

Comparison of the sequences of the clone 679 originating from P.falciparum clone T9/96, and of the corresponding sequence of LSA-3 originating from the isolate K1, shows that the gene is well conserved, the most significant differences being observed in the repeat region where the blocks of 4 amino acids are well conserved but vary in their number and organization.

In contrast, the non-repeat 5' and 3' portions appear to be especially well conserved, showing up to 100% homology in the 5' region where B and T epitopes have already been identified.

DNA amplifications, in particular by PCR of different P.falciparum strains with 8 primer pairs distributed over the whole of the LSA-3 gene, showed that, except with the ones surrounding the repeat regions, the whole of the genome gives PCR products of similar size, suggesting that the LSA-3 antigen is well conserved.

Various LSA-3 probes, chosen in the repeat and non-repeat regions, were hybridized at low stringency with the DNAs of different species of Plasmodium, and did not enable any gene homologous to LSA-3 to be identified except in the chimpanzee parasite P.reichenowi, confirming the close kinship of this species with P.falciparum.

Surprisingly, the antigen analogous to LSA-3 found in P.yoelii, which gives clear immunological cross-reactions at the surface of the sporozoite with antibodies against the fragment 729S, does not appear

to be conserved at the level of the nucleotide sequence. Lastly, comparison of the LSA-3 sequences with the data bases did not reveal any homology with known molecules, except for the repeat region, some of the motifs of which display a strong analogy with the repeats of a Staphylococcus xylosis gene, but also with two P.falciparum antigens, RESA and Pf11.1, which are both expressed during the blood stage of the parasite. This homology is essentially due to the large amount of "Glu-Glu" sequences in these antigens and in the repeats of LSA-3.

2) Cloning

The insert DG729 and other regions of exon 2 of the strain K1 were cloned into a prokaryotic expression vector pGEX, a vector marketed by the company InVitrogen Corp (San Diego USA). This vector produces a fusion protein with the Schistosoma mansoni glutathione S-transferase (GST), and enables the recombinant proteins to be purified readily by affinity for glutathione-agarose beads. The expression peptides from these vectors are designated:

- for the whole LSA-3 protein: REC protein,
- or for the fragment 729S:729PGEX.

Attempts at cloning other fragments, in particular the fragment 1-5 3NSREP, 3NFREP, 5NR and 5SNREP, caused difficulties related either to the cloning or to the production and purification of the proteins in sufficient amounts for immunization experiments.

Only the fragments 729, NN and 3PC enabled corresponding recombinant polypeptides to be produced and purified in sufficient amounts for analysis of the antigenicity of the molecule.

Example 2: Comparison of the antibody responses of chimpanzee Nuria before and after immunization with different peptides

Figure 5 depicts the amounts of immunoglobulins present in the serum of chimpanzee Nuria before and after immunization with the peptides 729NR1 and 729RE, and the lipopeptides 729NR2 and CT1.

This experiment shows the superiority as regards B immunity of the R antigen, most particularly when it is conjugated to a lipid residue.

Figure 6 shows that the level of specific antibodies measured by ELISA against the peptide 729NR2 in mice immunized with either the peptide 729NRII or the lipopeptide 729NRII is markedly higher when the lipopeptide is used, irrespective of the species of mouse.

Example 3: Effects of the antibodies against the LSA-3 peptides on the inhibition of the entry of sporozoites in mice

The techniques used to prepare the primary hepatocyte cultures, the sporozoites, the antibodies and the indirect fluorescence test are described in detail by S. Mellouk et al., Bulletin of the World Health Organization, 68: 52-59, 1990. The table below compares the results obtained in immunofluorescence, either with antibodies against the fragment 679 or with antibodies obtained against fragments originating from other peptides. The left-hand column shows the number of schizonts detected after 48 h of culture in hepatocytes of Balb/c mice infected by P.yoelii and the right-hand column the same parameters after infection by P.berghei.

Antibody clones	P.yoelii			P.berghei		
	IFA	No. of LS at 48 h		IFA	No. of LS at 48 h	
		a)	b)			
Control		88	110		119	108
679	++		0	-		47
	++		0	-		ND
679	++	1		-	105	
679b	++	1		-	133	
679c	++	1		-	30	
32	++	8		±	103	
222	+		5	±		26
667	++	276	143	ND	502	
362	+	3				
493	++	55		ND	508	
α P.b. CSP Mab			82	+++		30
α P.y. CSP Mab	+++		171		138	

It is clearly apparent that the antibody against the peptide 679 has an almost complete inhibitory effect on the number of what they [sic] observed at 48 h in the liver cells. Likewise, Figure 7 shows the inhibition of the sporozoite invasion of liver cells by hyper human [sic] sera obtained after immunization with different peptides and immunopurified against whole LSA-3.

Example 4: Cytotoxicity test against the peptide 729NRII in the chimpanzee Gerda

The chimpanzee Gerda was immunized via the i.v. route with the lipopeptide 729NRII originating from the LSA-3 antigen. Blood is drawn 9 days after the 4th injection. The PBMCs were incubated in vitro with 5 µg/ml of the peptide 729NRII (addition of recombinant IL-2, 10 U/ml, on day 3). On day 15, the cytotoxic activity was studied against autologous blasts generated with PHA at a concentration of 0.5 µg/ml. The blasts were preincubated overnight with 5 µg/ml of the peptide 729NRII, and with a control peptide, namely RESA, or without a peptide. The peptides are not added

during the test (8 hours). The number of targets per well is 5000.

PBMCs from Gerda incubated for the same period with 5 µg/ml of a control peptide or the peptide 729NRI (originating from the same antigen) do not bring about the lysis of autologous blasts preincubated or otherwise with the above peptides.

Figure 8 shows the results obtained for an E/T (effector to target) ratio varying from 12 to 0.03. It is seen that the target cells presensitized with the peptide 729NRII are lysed in the presence of effector cells, indicating a cytotoxic T type immune response specific to this antigen.

The lipopeptide NRII injected via the i.v. route is capable, without adjuvant, of inducing a specific cytotoxic response.

Example 5: Effect of the peptide NRI on interferon-γ production

Interferons have been shown to have an inhibitory activity in the development of P.falciparum in human hepatocytes in culture (Sylvie Mellouk et al., The Journal of Immunology, vol. 139 No. 12: 41-92, 41-95, 1987). The results obtained with the peptides of the invention are as follows:

The chimpanzee Gerda, immunized with the polypeptide NR2 and boosted with the recombinant DG729, carries PBMCs capable of secreting high levels of IFN-γ in the presence of the LSA-3 peptides, especially the peptide 729NRI. The result was confirmed in the chimpanzee Dirk, immunized with the same protein. The chimpanzee BRAM, an unimmunized control, does not show any interferon in the blood against the LSA-3 peptides.

The sequences ID n° 1 to 3 are depicted in figures 1 to 3.

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GTGATGAACT TTTTAATGAA TTATTAAA 28

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

TGTTGTTCTT GTTGAACACT TTTTACTAA 29

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

GGTATCGAAA CTGAGGAAAT AAAGG 25

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

CATAGCAGGA ACATCAACAT CCAC 24

CLAIMS

1. Polypeptide molecules containing at least 10 consecutive amino acids of the amino acid sequence shown in Figure 2, the following polypeptides being excluded:

- RDELFNELLNSVDVNGEVKENILEESQVNDDIFNSLVKSVQQEQQHNVEE
- VEESVEENDEESVEENVEENVENNDDGSVASSVEESIASSVDESIDSSIE-ENVAPTVEEIVAPTVEEIVAPSVVEKCAPSVEESVAPSVEESVAEMLKER (729S)
- RDELFNELLNSVDVNGEVKENILEESQVNDDIFNSLVKSVQQEQQHN
- DELFNELLNSVDVNGEVKENILEESQ, (NRI)
- LEESQVNDDIFSNSLVKSVQQEQQHNV, (NRII)
- VESVAPSVEESVAPSVEESVAENVESSV. (729RE)

2. Molecules according to Claim 1, characterized in that they contain at least 20 consecutive amino acids of the said sequence.

3. Molecules according to Claim 2, characterized in that they contain at least 50 consecutive amino acids of the said sequence.

4. Polypeptide molecule displaying at least 70% homology with one of the molecules of any one of Claims 1 to 3.

5. Polypeptide molecule, characterized in that it displays at least 70% homology with the following sequence:

Leu Leu Ser Asn Ile Glu Glu Pro Lys Glu Asn Ile Ile Asp Asn Leu Leu Asn Asn Ile (CT1).

6. Polypeptide molecule according to one of Claims 1 to 4, characterized in that it displays at least 70% homology with the sequence depicted in Figure 3.

7. Immunogenic composition, characterized in that it contains at least one polypeptide molecule according to any one of Claims 1 to 6 and at least one pharmaceutical vehicle.

8. Antimalarial vaccine composition containing, among other immunogenic principles, a polypeptide molecule according to one of Claims 1 to 6.

9. Vaccine composition according to Claim 8, characterized in that it contains, in addition, a molecule containing at least one epitope and which originates from the group consisting of the LSA-1, SALSA or STARP molecules.

10. Composition according to Claim 9, characterized in that it contains at least two immunogens, the first being chosen from the following polypeptides:

- that of Figure 2,
- NRI,
- NRII,

and the second being chosen from the group consisting of SALSA, SALSA I and SALSA II.

11. Polyclonal or monoclonal antibodies which specifically recognize the polypeptide molecules according to any one of Claims 1 to 6.

12. Method of in vitro diagnosis of malaria in an individual likely to be infected by P. falciparum, which comprises the bringing of a tissue or biological fluid taken from an individual into contact with a molecule according to one of Claims 1 to 8, under conditions permitting an immunological reaction, said polypeptide molecule and antibodies possibly present in the tissue or the biological fluid, and the in vitro detection of the antibody gene [sic] complexes possibly formed.

13. Method according to Claim 12, characterized in that the tissue or biological fluid is brought into contact with a mixture of polypeptide molecules responding [sic] to one of Claims 1 to 6 and other molecules originating from antigens of the sporozoite stage, namely LSA-1, SALSA or STARP.

14. Method of in vitro diagnosis of malaria in an individual likely to be infected by P. falciparum,

characterized in that it comprises the bringing of a tissue or biological fluid taken from an individual into contact with antibodies according to Claim 11, under conditions permitting an immunological reaction in vitro between the said antibodies and the proteins specific to P. falciparum which are possibly present in the biological tissue, and the in vitro detection of the antigen/antibody complexes possibly formed.

15. Kit for the in vitro diagnosis of malaria according to Claim 12 or 13, characterized in that it comprises at least one or several molecules according to one of Claims 1 to 6,
the reagents for making up the appropriate medium for the reaction,
the reagents enabling the antigen/antibody complexes produced by the immunological reaction to be detected, it also being possible for these reagents to carry a label or to be capable of being recognized in their turn by a labelled reagent, more especially in the case where the abovementioned polypeptide molecule is not labelled.

16. Kit for the in vitro diagnosis of malaria, characrerized in that it comprises:
- antibodies according to Claim 11,
- the reagents for making up the appropriate medium for carrying out the immunological reaction,
- the reagents enabling the antigen/antibody complexes produced by the immunological reaction to be detectd, it also being possible for these reagents to carry a label or to be capable of being recognized in their turn by a labelled reagent, more especially in the case where the abovementioned antibodies are not labelled.

17. Use of a polypeptide molecule according to one of Claims 1 to 6 in the preparation of an antimalarial vaccine.

18. Use of one or more polyclonal or monoclonal antibodies according to Claim 11 for the preparation of

a medicinal product intended for the treatment of malaria.

19. Pharmaceutical composition containing as active substance one or more polyclonal or monoclonal antibodies according to Claim 11, in combination with an acceptable pharmaceutical vehicle.

20. Nucleic acid sequence, characterized by one of the following sequences:

(a) the linked succession of nucleotides as depicted in SEQ ID No. 1 of Figure 1, or

(b) the linked succession of nucleotides depicted in SEQ ID No. 2 of Figure 2,

(c) a linked succession displaying at least 70% homology with that of Figure 1 or of Figure 2, or

(d) a linked succession of nucleotides which are complementary to those presented in (a), (b) or (c).

21. Nucleic acid according to Claim 20, containing a sequence coding for a polypeptide molecule according to one of Claims 1 to 6.

22. Recombinant vector for the cloning of a nucleotide sequence according to Claim 20 or Claim 21 and/or the expression of a polypeptide encoded by the abovementioned sequence, containing the said sequence in one of the sites which is not essential for its replication, the said vector being, in particular, of the plasmid, cosmid or phage type.

23. Vector according to Claim 22, characterized in that it is a plasmid deposited at the CNCM under the No. I-1573 and referenced pK1.2.